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ABSTRACTS OF TUBERCULOSIS

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Influence of Syphilis on Tuberculous Lesions.—There is a wide difference of opinion as to the part played by syphilis in tuberculosis. Some believe that syphilis predisposes to tuberculosis and creates a site of predilection for the tuberculous process; others that active syphilis influences unfavorably the course of tuberculosis. On the other hand a dissenting opinion holds that there is a definite antagonism between the two diseases or at least no significant clinical relation. In order to determine whether syphilis influences the course of tuberculous lesions, a study of the gross and microscopical character of tuberculous lesions of the skin of syphilitic and of nonsyphilitic rabbits was undertaken. Twelve rabbits were infected with *Tr. pallidum* by the testicular route. Nine weeks later an injection of 0.1 mg. of a virulent strain of bovine tubercle bacilli in 0.1 cc. of physiological salt solution was made into the abdominal skin at each of six widely separated points. At the same time 12 nonsyphilitic similar rabbits were injected in precisely the same manner with a like amount of the bacillary suspension. The resulting tuberculous lesions were examined and then subjected to biopsy for histological study according to the following scheme: In one series of 3 syphilitic and nonsyphilitic rabbits at varying intervals of from one to forty-eight hours after infection; in another series of from three to fourteen days and in the remaining rabbits the interval was weeks. A section of uninoculated skin was removed from each rabbit as a control. In these experiments the syphilitic rabbits reacted in a different manner to the injection of living virulent tubercle bacilli than did similarly infected nonsyphilitic rabbits. In the syphilitic rabbits the local inflammatory reaction following the injection of tubercle bacilli into the skin was more intense in character, as evidenced by the greater degree of oedema and cellular infiltration about the widely dilated vessels. As early as three hours after infection, and persisting throughout the period of observation, it was noted that in the syphilitic rabbits the lesions were multiple, focal in character and distributed about the capillaries, whereas in the nonsyphilitic rabbits the lesion was single, diffuse in character and bore no relation to the vascular distribution. Histologically the lesions removed at intervals of forty-eight hours to three weeks following the injection of the tubercle bacilli into the syphilitic rabbits consisted of perivascular aggregations of large mononuclear cells, fibroblasts and newly formed capillaries. These lesions resembled in their histological characteristics the primary and secondary lesions of syphilis rather than the characteristic lesion of tuberculosis, although scrapings from more than 200 such lesions examined by means of dark-field have failed to show the presence of *Tr. pallidum*. Epithelioid cells, which were observed in both syphilitic and nonsyphilitic rabbits about two weeks after infection, were fewer among the syphilitic rabbits and perivascular in their distribution, replacing the large mononuclear cells. On the other hand, among the nonsyphilitic rabbits the epithelioid cells

appeared in large numbers scattered diffusely throughout the tissue. In the syphilitic rabbits fibroblasts and newly formed capillaries made their appearance within forty-eight hours after infection with tubercle bacilli and increased rapidly in number, with the formation of dense bundles of connective tissue which separated the lesions, while in the sections removed from the non-syphilitic rabbits there was a paucity of fibroblast formation and an absence of capillaries. There is considerable evidence showing that animals, previously immunized but having subsequently reached a relatively antibody-free stage, will, when injected with an unrelated or remotely related antigen, respond with the production not only of antibodies against the last antigen, but also with the production of antibodies against the initial antigen. This revived production of antibodies to the original antigen, Bieling has termed the "anamnestische Reaktion." There is less evidence to indicate that this same type of "anamnestische Reaktion" is involved in the cellular response to an irritant. However, that the application of a caustic to the skin of latent syphilitics produced characteristic local syphilitic lesions has been noted. These observations on the cellular response of syphilitic rabbits to the injection of virulent bovine type tubercle bacilli suggest that the reaction is of the "anamnestische" character and that the cells of the syphilitic rabbits are so modified that the introduction of an unrelated organism elicits a prompt inflammatory reaction characteristic of the initial syphilitic infection. The perivascular focal character of the lesion, the presence of large mononuclear cells and fibroblasts, and the formation of new vessels suggest syphilis; on the other hand the subsequent appearance of epithelioid cells and of caseation and softening are more characteristic of tuberculosis.—*The Effect of Syphilis on Local Tuberculous Lesions in Rabbits, J. D. Aronson & D. R. Meranze, Am. J. Path., March, 1938, xiv, 163.*—(*J. S. W.*)

Influence of Primary Disease on Experimental Tuberculosis.—Whether a primary tuberculous infection should be considered as affording protective value against reinfection or as a state carrying with it something which predisposes to more extensive tuberculosis or reinfection is still a moot question. The purpose of the experiments reported in the present article was to determine the effect of primary pulmonary tuberculosis (BCG) in the left lung on the progress of tuberculosis in the right. In a series of rabbits a nodular mass or primary tubercle was produced by injecting 2 mg. of living BCG through the thoracic wall and pleural space into the lower lobe of the left lung. A lesion comparable to a primary focus can always be produced by this method. Pleurisy or extension in the lung or to another lobe or elsewhere does not occur. Three weeks after the inoculation with BCG all rabbits were tested with OT intracutaneously. Nearly all animals showed some degree of allergy. Thirty-two normal rabbits and 38 animals with primary BCG tubercles were then (forty days after BCG) infected by injecting 0.01 mg. of a virulent strain of bovine tubercle bacilli into the lower lobe of the right lung. Animals in both groups were killed for examination at periods after the injection of virulent bacilli ranging from one to one hundred and ten days. The amounts of tuberculosis present in the right lung of both groups and the size of the primary lesion and the degree of allergy previously present in the immunized animals are shown in a table. In the periods of from one to seven days (animals examined after one, two, four and seven days) tubercles appeared much earlier in the allergic than in the normal animals. At from nine to fifteen days tuberculosis was noted to be progressing in the normal animals and to be retarded in the allergic ones. From twenty-eight to fifty-one days there was extensive tuberculosis in the normal animals, while most of the allergic animals showed retardation or absence of tuberculosis, not only at fifty-one days, but for as long as one hundred and ten days after the virulent tubercle bacilli were injected into their right lungs. The degree of allergy and the size of the primary pulmonary lesion in the left lung seemed to bear no relation to each other or to the degree of tuberculosis in the right lung. These experiments illustrate the influence of an existing inactive primary pulmonary tuberculous lesion on a subsequent exogenous infection. The protection observed cannot be attributed to allergy alone, for in previous experiments the author found that a marked degree of resistance could be produced artificially both locally and generally in the absence of allergy. The quick inflammatory response, on the other hand, was probably due, to a great extent, to existing allergy, since the local tissue response has been found to be greater in allergic immune animals than in nonallergic immune animals. It appears evident that the allergy which was associated with other mechanisms of defense in these animals can be considered a factor in resistance by hastening the phenomenon of inflammation and bringing the phagocytic cells in contact with the living organisms more quickly. While allergy may without

question destroy tissue in some cases to the extent of spreading the infection, it seems, on the other hand, that assistance in resistance may be afforded by the allergic phenomenon in bringing about the tissue defense more quickly. These experiments suggest that a retarded or healed primary pulmonary tubercle may, in most cases, have a beneficial aspect which far overbalances the deleterious effects of the allergy present.—*Effect of a Primary Pulmonary Tuberculous Lesion (BCG) on Experimental Pulmonary Tuberculosis in Rabbits, B. J. Clawson, Arch. Path., November, 1938, xxvi, 966.*—(*J. S. W.*)

Spread of Tubercle Bacilli.—The present experiments were undertaken to determine whether the retardation of spread of tubercle bacilli in immunized animals is due to the local destruction of the organisms or to fixation at the site of injection. As this phenomenon might be influenced by the degree of virulence of the infecting organisms the experiments were performed with both highly and slightly virulent strains of tubercle bacilli. Havana rabbits were used and certain animals received two initial intracutaneous injections of heat-killed tubercle bacilli, followed by five single injections at weekly intervals. Each dose consisted of 0.1 mg. of bacilli suspended in 0.1 cc. of water. One week after the last injection all rabbits reacted to 20 per cent OT administered intracutaneously. Later 0.01 mg. of living tubercle bacilli was injected into the skin of these rabbits and their appropriate normal controls. Two series received a virulent bovine strain, another a moderately virulent human strain, and the final series a human strain of tubercle bacilli of low virulence. At selected intervals, ranging from three hours to one or more days, a normal and an immunized rabbit were killed. The infected skin areas and the inguinal lymph nodes were removed, ground, treated with oxalic acid and cultured. The approximate number of living organisms in the infecting dose had already been determined by culture. Thus it could be roughly determined whether there was an increase in the number of organisms present at the site of inoculation at any time following injection. In preliminary experiments it was found that the number of viable tubercle bacilli recovered from the skin immediately after the injection was less than the number injected. Portions of the spleen and lung tissue were also treated in a similar way and cultured. Sections for microscopical examination were prepared from the lung, liver and kidney of all rabbits, and from the spleen when not cultured. The number of bovine tubercle bacilli recovered from the skin was greater in the immune rabbits than in the normals up to the fifth day. Thereafter the skin of the normals usually showed more bacilli. Superficial necrosis with possible loss of tubercle bacilli occurred in the immune rabbits after five days as compared with nine days in the normals. A total of 2,721,860 organisms was recovered from the normal and 1,339,960 from the immunized rabbits. More tubercle bacilli were recovered from the inguinal lymph nodes of normal rabbits than from the comparable immunized ones and multiplication of bacilli occurred definitely earlier. In the series of rabbits receiving moderately virulent human tubercle bacilli more tubercle bacilli were recovered from the skin of immunized than from the skin of normal rabbits in all save one instance, and a considerable multiplication had apparently occurred in the skin within two days after injection. In the group receiving human bacilli of low virulence a similar result was obtained, but the difference between the immunes and the normals was not so marked. In general it can be said from these experiments that tubercle bacilli injected into the skin of rabbits immunized (sensitized) by heat-killed tubercle bacilli multiply within the first few days after injection, and are, after the same intervals, more abundant than in the similarly infected controls, if the loss by sloughing is taken into consideration. The superficial necrosis which occurred usually earlier and more severely in the immunized animals may have resulted in many tubercle bacilli being discharged when sloughing occurred. In the immunized animals the tubercle bacilli reached the draining lymph nodes from two days to two weeks later than those similarly injected into normal animals. Tubercle bacilli multiply actively at the site of injection during the period of retarded lymphatic dissemination; hence the retardation is caused by local fixation and not by destruction of tubercle bacilli. It is not improbable that both inflammation and antibodies are concerned in the fixation of antigens at the site of their injection. The combination of antigen and antibody doubtless causes the intense inflammation of the sensitized animal. Precipitation of soluble antigens or agglutination of bacteria produces aggregations that are not readily carried away from the site of the injection.—*The Spread of Tubercle Bacilli in the Bodies of Sensitized and Immunized Animals, J. Freund & D. M. Angevine, J. Immunol., October, 1938, xxxv, 271.*—(*J. S. W.*)

Cellular Reactions to Defatted Bacilli.—Living tubercle bacilli were treated with water and alcohol-ether and the residue from

this with chloroform. The material insoluble in chloroform, the second residue, consisted predominantly of defatted bacilli. Treatment of this residue with 25 per cent alcohol resulted in a third residue which was then treated with hydrochloric acid and extracted with ether-chloroform. This resulted in a fourth residue which was predominantly of non-acid-fast material. The present study involves the biological reactions to the lipids and polysaccharides from the second residue and the subsequent residues and extractives. Rabbits were inoculated intraperitoneally with the different materials. The findings are reported in tabular form and are difficult to describe on account of their complexity. *Summary:* The cellular reactions to defatted tubercle bacilli are complex and like those to heat-killed whole tubercle bacilli. The firmly bound lipid, when removed from these organisms, is non-acid-fast; it contains an hydroxy-acid which is acid-fast and a polysaccharide, which is not. This hydroxy-acid gives rise to foreign body giant cells and the tissues eventually become infiltrated with eosinophiles. The polysaccharides, both from the defatted bacilli and from the unfilterable lipid, call neutrophiles from the blood-stream. The reactions to the unfilterable lipid include those of both its constituents.—*Cellular Reactions to Defatted Tubercle Bacilli and Their Products* (*with two plates*), *F. R. Sabin & A. L. Joyner, J. Exper. Med., December, 1938, lxviii, 853.*—(*J. S. W.*)

Reactions Caused by Tuberculoprotein and Tuberculophosphatide.—In a previous report it was noted that the intracutaneous injection of tuberculophosphatide produced a local reaction in tuberculous animals more closely resembling the Koch phenomenon than a tuberculin reaction. In the present paper the cytology of this reaction is studied and compared with the response elicited by tuberculoprotein in tuberculous animals. The tuberculoprotein used was the water soluble MA-100 from human tubercle bacilli. The tuberculophosphatide, for the most part, was a highly purified preparation. Normal and tuberculous guinea pigs were inoculated with these substances. Forty mg. of the phosphatide (two preparations) and 1 mg. of the protein were injected subcutaneously. The phosphatide gave similar reactions in both groups of animals, but the response was quantitatively greater, and qualitatively more rapidly evolving in the tuberculous animals, and also was characterized by more intense and persistent reaction of polymorphonuclears, by more necrosis and by haemorrhage. In the protein injected animals, rapid reactions resulted in the tuberculous groups but no palpable reaction in the normal animals. Thus, while the doses of the phosphatide and protein given produced reactions of comparable and almost equal maximum size in the tuberculous animals, the phosphatide caused a definite reaction in normal animals whereas the protein did not. Guinea pigs inoculated intraperitoneally with phosphatide failed to react to MA-100 intracutaneously except after repeated doses. This was interpreted not as tuberculin hypersensitiveness initiated by the phosphatide but as a result of the repeated test injections of the tuberculoprotein. Microscopically the response to the phosphatide in normal animals is essentially a reaction of the monocytes which engulf the lipid and in so doing become epithelioid cells. In the tuberculous animals the cytological reaction is similar, but accelerated and characterized throughout by the presence of numerous neutrophiles. The greater response in tuberculous animals is very likely due to a modification of the connective tissue of the entire body, possibly nonspecific. The cellular response to the protein in tuberculous animals is frankly a neutrophilic one. In normal animals the response is very slight, disappearing in a few days. The reaction which the phosphatide characteristically elicits is not due to protein accompanying or bound to the lipin since the protein in normal animals causes little or no reaction and no epithelioid cells whereas the phosphatide induces a larger reaction characterized chiefly by epithelioid cells.—*Reactions of Normal and Tuberculous Animals to Tuberculoprotein and Tuberculo-phosphatide* (*with one plate*), *K. C. Smithburn & F. R. Sabin, J. Exper. Med., November, 1938, lxviii, 641.*—(*J. S. W.*)

Cellular Reactions to Tuberculoproteins.—The effect of the injection of tuberculoprotein given both in solution and in suspension is reported. All the proteins used for the study were derived from bacilli grown on a liquid synthetic medium entirely free from protein. Hence there was no contamination with any other protein. Normal rabbits were given intraperitoneal injections of a soluble form, a freshly precipitated form and relatively insoluble form of tuberculoprotein and the cell responses studied. Also a group of tuberculous guinea pigs received tuberculoprotein in two forms, in solution and in suspension. The results are reported in detail in tabular form. In contrast with the lipids, which induce relatively simple and uniform reactions, the proteins induce much more complex reactions because they may give rise not onlv to one form of the

phagocytic mononuclear cells, but in some cases to every form and even produce tubercles and giant cells. The degree of complexity of these reactions varies with the state of the protein, being simplest when caused by the protein in solution, more complex by the freshly precipitated proteins and most complex by the more insoluble forms. Tuberculoprotein in solution induced the formation of monocytes in normal animals and tubercles of epithelioid cells in tuberculous animals. Freshly precipitated tuberculoproteins induce a moderate formation of epithelioid cells in normal animals and more in the tuberculous. Insoluble forms of tuberculoprotein induce complex tubercular tissue in normal animals. This action is enhanced in the tuberculous.—*Cellular Reactions to Tuberculo-Proteins Compared with the Reactions to Tuberculo-Lipids (with two plates), F. R. Sabin, J. Exper. Med., December, 1938, lxviii, 837.—(J. S. W.)*

Attraction of Leucocytes by Tuberculoprotein.—Various microörganisms, including the tubercle bacillus, have the power of attracting human polymorphonuclear leucocytes. The chemotactic response of leucocytes to bacterial fractions, however, has not been extensively investigated. The present experiments have to do with the effect of tubercle protein on human leucocytes. The effect of nonspecific substances can be avoided by employing an *in vitro* method and this readily permits the use of human polynuclear leucocytes. The tuberculoprotein utilized was obtained from tuberculin by complete saturation with ammonium sulfate. A minute amount of this protein was placed on a clean glass slide as the target and this was then covered with a thin, evenly spread film of fresh human blood. The preparation was sealed and incubated at 37°C. After from fifteen to thirty minutes the preparation was placed under the microscope with a portion of the "target" appearing at the edge of the field, and the course of each migrating leucocyte was plotted on paper with the aid of a drawing ocular. Observations were made at intervals of one minute for a period of thirty minutes. For a control, the motions of the leucocytes in another field away from the actual target were recorded. The degree of chemotropism for any particular cell was figured at the ratio between the net approach of the cell either toward or away from the target to the actual distance traveled by the cell. In eight experiments in which 80 cells were observed the tuberculoprotein in its solid form was found not to attract the neutrophiles. Because in this experiment the protein presumably did not dissolve in the blood plasma, another series of tests was made in which the tuberculoprotein was dissolved before being used as the chemotactic substance. A one per cent solution of the protein was prepared presumably in the form of a sodium salt. In order to see the protein solution under the microscope it was necessary to adsorb it on some inert substance which could be used as a target. Washed kaolin or charcoal was selected for this purpose as they are relatively chemotactically inert. In this adsorbed state the protein exhibited strong positive chemotropism in contrast with the weak chemotactic properties of the pure adsorbing agent.—*Attraction of Human Polymorphonuclear Leukocytes by Tuberculoprotein, W. B. Wartman, Arch. Path., September, 1938, xxvi, 694.—(J. S. W.)*

Vitamin C in Tuberculosis.—A significant and progressive hypovitaminosis C was demonstrated by urine examinations during experimental tuberculosis in the guinea pig. This deficit was rapidly overcome by the daily oral administration of 10 mg. of l-ascorbic acid. A significant vitamin C deficit occurred in the suprarenals during experimental tuberculosis in the guinea pig and was completely overcome during the early infection by the oral administration of l-ascorbic acid.—*The Rôle of Vitamin C in the Pathogenesis of Tuberculosis in the Guinea Pig. I. Daily Excretion of Vitamin C in Urine of l-ascorbic Acid Treated and Control Tuberculous Animals. II. Vitamin C Content of Suprarenals of l-ascorbic Acid Treated and Control Tuberculous Guinea Pigs, K. E. Birkhaug, Acta tuberc. Scandinav., 1938, Fasc. 2–3, xii, 89.—(F. B. S.)*

Classification of Acid-fast Bacteria.—In the previous report (Gordon) an attempt was made to classify 252 strains of nonpathogenic mycobacteria. As 84 per cent of these cultures had been isolated from the soil, the question arose whether cultures from sources other than the soil would fall into the same groups and whether the standards previously set would be adequate. Seventy-nine more acid-fast cultures from cases of human and rat leprosy, bovine tissue, sputum, fish, water and soil were studied by the same methods. Only two of the three groups reported in the former study were represented in the more recent collection; as none of the cultures were of the *Mycobacterium phlei*, or group III type. The cultures were grouped according to a plan which differed slightly from the first arrangement. Group I: Fails to survive 60°C. for one hour. Grows at 47°C. (a) Utilizes arabinose, sorbitol, galactose, trehalose, mannitol, and

fructose. Fifteen strains similar in appearance and cultural reactions. (b) Unable to utilize all the above carbohydrates. Two strains differing in appearance and cultural reactions from the above cultures and from each other. Group II: Fails to survive 60°C. for one hour. Does not grow at 47°C. (a) Unable to utilize sorbitol. (*1*) Unable to utilize arabinose. Forty strains similar in appearance and cultural reactions. Two dissimilar strains. (*2*) Utilizes arabinose. Two similar strains. (b) Utilizes sorbitol. Eight dissimilar strains. (c) Unable to utilize any of the carbohydrates tested. Ten similar strains, sixty-five cultures (82 per cent) were readily assigned to closely related groups. The remaining 14 strains were arbitrarily distributed among four heterogeneous subgroups. The source and grouping of all the strains of saprophytic acid-fast cultures are given in one table.—*The Classification of Acid-Fast Bacteria. II, R. E. Gordon & W. A. Hagan, J. Bact., July, 1938, xxxvi, 39.—(J. S. W.)*

Synthetic Culture Medium.—The cloudiness observed in the synthetic culture medium D, devised by Lockemann for the isolation and cultivation of tubercle bacilli was obviated by substituting magnesium sulphate for magnesium citrate. Iron, either in the form of ferrous or ferric salts, was found to enhance the growth of tubercle bacilli. The addition of 1 mg. of iron per litre of solution yielded 14 per cent more growth after four weeks' incubation than the cultures grown on an iron-free medium. After six weeks' incubation the human type yielded 39 per cent more growth and the bovine 82 per cent more than the control cultures grown on an iron-free medium. Amounts of iron larger than 1 mg. per litre were found to give less growth.—*Zur Frage der eiweissfreien Nahrlösungen für Tuberkelbazillen, G. Lockemann, Zentralbl. f. Bakt., July, 1938, cxlii, 79.—(J. D. A.)*

Preserving Contaminated Pathological Products.—The following method was devised to eliminate the contaminating microörganisms in pathological products to be examined for the presence of tubercle bacilli. The pathological material was placed in five to ten times its volume of a solution of 10 per cent carbonate of soda. The tissue fragment must not be more than 3 to 4 cm. in thickness. If the product be liquid, such as pus, exudate, urine or milk, it was diluted about one-half its volume with the carbonate solution. It was then placed in a sterile container hermetically sealed for shipment. Under these conditions the development of secondary organisms was arrested and even destroyed after a few hours. Fragments of a large intestine placed in the solution of carbonate of soda have been preserved at room temperature for several weeks without any trace of putrifaction. The carbonate solution was tested for its possible bactericidal effect upon tubercle bacilli. A measured number of bacilli were placed in the 10 per cent solution of sodium carbonate and also in Ringer-Locke solution as a control. These were left at room temperature and in the ice box from twenty-four hours to eight days. It was shown that the soda had practically no inhibitory effect upon the tubercle bacilli. This method is recommended for preserving pathological material for subsequent examination for living tubercle bacilli.—*Procédé de conservation des produits pathologiques en vue de la culture du bacille tuberculeux, R. Laporte & R. Grimal, Compt. rend. Soc. de biol., May 7, 1938, cxxviii, 42.—(M. T. W.)*

Staining of Acid-fast Bacilli in Paraffin Sections.—Alcohol is the fixative of first choice for purposes of staining acid-fast bacilli in paraffin sections. However, it causes much shrinkage around the margins of the piece of tissue, not such a great disadvantage with large fragments but noticeably so when small pieces of tissue are to be sectioned. The addition of 10 per cent formaldehyde to the alcohol improves the fixative without affecting the staining of the bacilli. The use of formaldehyde alone is not satisfactory although brief fixation in neutral formaldehyde solutions, while not ideal, may be permissible. The disadvantages of fixation in Orth's, Mueller's or Zenker's fluid can largely or completely be overcome by thoroughly washing in distilled water to remove the fixing fluids, but it is doubtful if tissues so treated ever measure up to tissues fixed in alcohol for purpose of staining acid-fast bacilli. The classical method of removing the mercury deposits from the tissues with Lugol's iodine-potassium iodide mixture is wholly satisfactory, but, the iodine must be completely removed. Removal of the iodine by alcohol is often a slow process; conversion by sodium thiosulphate into sodium iodide is very rapid, but tends to leave water-insoluble deposits which will precipitate fuchsin. It seems best, therefore, to treat the section with both alcohol and sodium thiosulphate (in this order) to avoid all difficulties. In the author's experience trimethylated pararosaniline (with the methyl group attached directly to the phenol ring) or new fuchsin possesses much greater powers of staining acid-fast bacilli than the pure simpler compounds of basic fuchsin itself. It imparts a

somewhat darker, less red and more magenta tint than does rosaline, but the dyed bacilli display greater sharpness of outline and deeper staining. The staining combination found most effective consisted of new fuchsin in a one per cent solution in 5 per cent phenol and 10 per cent methyl alcohol. For decolorization the classical 1 to 5 per cent hydrochloric acid in alcohol (70 to 95 per cent) is wholly satisfactory. The lower strength has nearly the same decolorizing effect as the higher, and is preferred. When used separately much stronger acids are employed, for acid is much more effective in alcoholic than in aqueous solutions. Probably the simplest counterstain is methylene blue in 0.1 to 1 per cent solution rendered very slightly alkaline with a few drops of ammonia. To insure maximal staining after alcohol fixation the sections should be stained from two to eight hours at 20°C., one to four hours at 37°C., thirty minutes to two hours at 60°C., and five minutes at 90°C. (steaming). After all other fixations the time should be three or four times longer except when steaming. Prolonged staining at room temperature is preferred. New fuchsin as a stain for acid-fast bacilli is much superior to basic fuchsin.—*The Staining of Acid-fast Bacilli in Paraffin Sections, G. L. Fite, Am. J. Path., July, 1938, xiv, 491.*—(*J. S. W.*)

Serological Differentiation of Acid-fast Bacteria.—The differentiation of tubercle bacilli of human, bovine, avian and frog origin, as well as some acid-fast saprophytes, was carried out by means of agglutination and agglutination-absorption experiments. Rabbits undergoing immunization with these acid-fast bacteria frequently succumbed to pneumonia after several injections, while some strains of the avian tubercle bacillus proved highly toxic to the animals. Anti-timothy bacillus serum proved highly specific, and gave no cross reactions with the various types of tubercle bacilli. The serum prepared with the frog strain gave, in high concentration, cross reactions with the human and avian tubercle bacilli, while the serum prepared with human type tubercle bacilli, in high dilution, agglutinated the frog strain. While antigenic specificity between acid-fast saprophytes and pathogenic acid-fast bacteria could be demonstrated by agglutination, similar results were not obtained by means of the complement fixation reaction. It was found that one strain of human tubercle bacillus produced immune serum which gave some cross agglutination with avian bacilli, while with immune serum produced by another human strain it failed to do so. However, both sera agglutinated bovine tubercle bacilli. Agglutination of avian bacilli, except R variant, occurred promptly; the agglutinated mass was flaky in character, and could readily be broken up. Agglutination of the mammalian types and the R variant of the avian type occurred slowly; the mass was granular in character and the clumps were not readily dispersed upon shaking. Differences in the antigenic constitution of avian and mammalian tubercle bacilli were proved by agglutination-absorption experiments. It was found that avian bacilli failed to remove the agglutinins for the mammalian type from immune serum prepared by the injection of human type tubercle bacilli. Similarly, mammalian type tubercle bacilli failed to remove the agglutinins for the avian type from specific immune serum. That the antigenic constitution of different strains of avian tubercle bacilli differs was shown by means of the agglutination-absorption studies. The author concludes that the antigenic structure of the mammalian type of tubercle bacilli differs from that of the avian type, and that different strains of avian tubercle bacilli differ in their antigenic structure.—*Versuche einer Differenzierung der säurefesten Bakterien mittels des Agglutinationsverfahrens, C. Guglielmo, Zentralbl. f. Bakt., September, 1938, clxii, 303.*—(*J. D. A.*)

Mixed Infections with Human and Bovine Tubercle Bacilli.—In a series of 5,000 cases of tuberculosis in Denmark, 14 were found to be mixed infections, and in 100 cases under observation for a year, only 2 such cases were found. During a period of one to four years, in which 17 cases were examined serially, 9 were found to become mixed infections. Bovine strains of tubercle bacilli during their stay in the human organism or during cultivation for a brief period are modified into strains which, in their characters of growth and virulence, approach the human type so closely that present available methods cannot distinguish between them. The questions whether the bovine type bacillus is transformed into the human type and whether the figures for the occurrence of the bovine infection give only a minimum of cases are considered. Extensive protocols are given and a description of the technique used for isolation and identification of the strains is presented.—*Studies on the Types of Tubercle Bacilli Isolated from Man. V. Mixed Infections with Human and Bovine Tubercle Bacilli, K. A. Jensen & I. Kiaer, Acta tuberc. Scandinav., 1938, Fasc. 2-3, xii, 105.*—(*F. B. S.*)

Separation of Mammalian Tubercle Bacilli.—The author suggests that the human and bovine types of tubercle bacillus may be either different phases of the same organism or that the two types may be symbiotic. Pure types of one or the other are usually unobtainable. Forty-eight strains of tubercle bacilli from human sources and 20 from bovine sources were planted repeatedly upon Löwenstein's medium free of dye, as well as on Sauton's medium, broth and synthetic medium ranging in pH from 5.6 to 8.0. Single colonies were studied and transplanted for from 5 to 15 generations. The author describes two types of colonies. Type 1 appears as a fine film-like colony, about the size of streptococcus colonies, which frequently cannot be seen by the unaided eye. These colonies are grayish-white, warty and slow-growing. After several months of incubation a gray, granular, fine, flat, filmy growth may be noted about the original colony. In this area the human type of bacillus may be obtained in pure or almost pure state. Morphologically these organisms appear as threads with numerous branching forms; they are nonpathogenic for guinea pigs and rabbits. Type 2, which represents the bovine type, appears as glassy, bluish-white, shiny, hemispherical colonies. These colonies consist in most instances of bovine bacilli; occasionally they contain a small number of human bacilli. The human type 1 colony fails to grow when reinoculated, unless it contains a small number of the bovine bacilli, while the bovine type 2 colony gives good and rapid growth when reinoculated, and the rate of growth increases in subsequent generations. The author attributes the loss of pathogenicity noted in the type 1 colony to the absence of bovine type bacilli. The human type grows best on solid media of neutral or slightly alkaline reaction; the rate of growth and thickness of pellicles are greater in the neutral and alkaline range, but they are inhibited at pH 8.0. The pellicle is grayish-yellow or pale yellow in color. On solid media having a neutral or slightly alkaline reaction colonies of the bovine cultures differ from the original colonies in that they are larger and higher and tend to dissociate, on the one hand, into typical colonies possessing the characteristics previously described and, on the other, into colonies which are dry, rough and variable in color from yellow to orange-red and brick-red. The typical bovine colony is found to consist of almost pure bovine bacilli, while the rough atypical colonies are found to have an increasing number of human bacilli. The author concludes that the vitality and pathogenicity of the human type tubercle bacillus is dependent upon the presence of bovine bacilli, and that a small number of human bacilli are found associated with the bovine type.—*Ueber Versuche zur Trennung gemischter Tuberkelbazillenkulturen auf Nährboden von verschiedenem Alkalitätsgrad, G. Eduard, Zentralbl. f. Bakt., August, 1938, clxii, 190.*—(*J. D. A.*)

Cultural Differentiation of Avian Tubercle Bacilli.—The cultural characteristics of avian type tubercle bacilli grown on Besredka's egg medium and on Lockemann's synthetic medium containing from 20 to 50 per cent rabbit serum were compared with the cultural characteristics of the human and bovine types of tubercle bacilli and eight different acid-fast saprophytes. It was found that in these liquid media the avian strains usually produced a slimy, stringy sediment which upon shaking presented a cotton-like appearance and diffuse cloudiness throughout the medium. Pellicle formation was absent in these cultures. On the other hand, the mammalian type tubercle bacilli and the acid-fast saprophytes produced a pellicle on the surface of the culture media, while the underlying liquid remained clear and, usually, no slimy sediment was noted. The slimy sediment failed to appear in one of eight avian strains and in an R variant of an avian strain. On the other hand, one of five bovine strains produced the slimy sediment.—*Gelingt es, Tuberkelbazillen des Typus gallinaceous nach ihrem Wachstum in flüssiger Kultur von anderen Säurefesten sicher zu unterscheiden, C. Guglielmo, Zentralbl. f. Bakt., September, 1938, cxlii, 313.*—(*J. D. A.*)

Thermal and Chemical Resistance of Tubercle Bacilli.—The authors, in an earlier paper, studied the thermolability of the tubercle bacillus and noted a high resistance to temperatures affecting other microorganisms detrimentally. Occasionally a test, however, would give a survival time differing from that found in the ordinary run of experiments. The present article represents further observations on the mechanism of this variation in the cultures of the bacilli. At the same time the resistance of human tubercle bacilli, obtained from various mediums, to 2.5 per cent acetic acid was studied. It was found that the type of medium on which the bacilli had been cultured was a determining factor in the resistance of these bacilli. Those cultured on egg media containing broth usually showed a greater resistance to heat and acetic acid

than those cultured in the absence of broth. Likewise, bacilli cultured on whole-egg media with broth showed a greater resistance than those cultured on glycerol broth agar. The age of the culture, within certain limits of from three to six weeks, appeared to exert no appreciable effect on the resistance of the bacilli. There was no apparent relation between the ability of a medium to support the growth of small numbers of tubercle bacilli and thermal lability or resistance to acetic acid. The use of varying types of media, besides errors in technique, probably account for the wide variations in thermal resistance noted for tubercle bacilli in the literature. The thermal survival time of avian tubercle bacilli exceeded that noted for human tubercle bacilli under the same conditions of culture and heat exposure.—*Some Fundamental Investigations on the Resistance of Tubercle Bacilli, H. J. Corper & M. L. Cohn, J. Bact., March, 1938, xxxv, 223.—(J. S. W.)*

Differentiation of Human and Bovine Tubercle Bacilli.—It has been demonstrated that, when given in proper doses by suboccipital inoculation into the rabbit, tubercle bacilli of the human type show a difference in virulence from tubercle bacilli of the bovine type. Usually the human tubercle bacilli are completely avirulent while the bovine strains of bacilli produce a fatal meningitis. This method of inoculation makes a simple, rapid and certain means of differentiating the two types of bacilli. A series of experiments was made to bring out these points, inoculating pure cultures of the two types of bacilli suboccipitally. Seven strains of bovine tubercle bacilli and sixteen strains of human bacilli were inoculated in 0.01 mg. doses into the meninges of rabbits; all animals developed paralysis toward the second week following the infecting inoculation, and they were dead two to five days later with meningitis. Suboccipital inoculations of human strains of tubercle bacilli generally did not affect the rabbits with the exception of two abnormally virulent strains. After the seventh week the rabbits which had received these strains developed paralysis which progressed slowly into a fatal meningitis several weeks later. The remaining rabbits inoculated with the human strains of tubercle bacilli, dead or sacrificed sixty to one hundred and sixty days after the inoculation, showed no symptoms of meningitis.—*Détermination du type de bacilles tuberculeux des mammifères par inoculation intraméningée au lapin, A. Boquet, Compt. rend. Soc. de biol., February 19, 1938, cxxvii, 575.—(M. T. W.)*

Diminution of Virulence of Strains of Avian Tubercle Bacilli.—Reports have previously been made of the loss of virulence of an R variant of a strain of avian tubercle bacilli while the S variant of the same strain retained its initial high virulence even after five years of passage *in vitro*. The diminution of virulence of avian strains of tubercle bacilli may not always be brought about by the same mechanism. The following report is of the diminution and loss of virulence of avian tubercle bacilli which occurred while the organisms retained all their cultural characteristics and properties of true avian bacilli. The first strain was isolated from the bone marrow of a spontaneously infected chicken. It was culturally a typical avian tubercle bacillus with characteristic and high virulence for susceptible laboratory animals. The typical S colonies were kept on potato-glycerine medium for a period of four years at which time the original S aspect of the culture had been maintained. At this time the virulence of the organism for the chicken had decreased to such a point that doses of 0.01 mg. were required to produce a light infection in chickens sacrificed five months after inoculation. In this case it appeared that the successive transplants on potato-glycerine medium over the four-year period was responsible for the loss of virulence. The original culture kept on egg medium over the same period of four years had retained its initial virulence for the chicken. The second strain reported had been isolated from a chicken and kept for a period of six years. It retained its original S character on various culture media. When tested for virulence a year after isolation a dose of 0.000,01 mg. was sufficient to kill a chicken. At the end of five more years of passage a dose of 0.1 mg. was required to kill a chicken. These results may be added to some results previously published of the study of two strains of avian tubercle bacilli used for twenty years for making avian tuberculin. These two strains, even after twenty years of transplants, were still typical S cultures, but massive doses of 0.1 mg. failed to infect chickens. These experiments show that certain strains which were kept for many years in the course of numerous passage *in vitro* lost virulence. The importance of these studies lies in the clearly demonstrated fact that the loss of virulence of avian strains may be shown in two S variants as well as in the R dissociates. Because of this diminution of virulence of two variants, the dissociation of the avian tubercle bacillus becomes entirely comparable to that which has been observed in other species of micro-organisms.—*Diminution de virulence de la*

variante S de deux souches de bacilles tuberculeux aviaires, après des années de passages in vitro sur la pomme de terre glycérinée, A Saenz, Compt. rend. Soc. de biol., January 22, 1938, cxxvii, 186.—(M. T. W.)

Pathogenic Properties of S and R Avian Tubercle Bacilli.—The question of pathogenicity of the S variant of avian tubercle bacilli presents, outside of a practical importance, a theoretical importance in facilitating the explanation of the phenomenon of dissociation. Numerous experiments made by various investigators have shown that in general a passage of the smooth form into the rough variant is accompanied by a decrease of the pathogenic properties. However certain R variants coming from recently isolated strains did retain their virulence. Studies on R and S variants of five strains of avian tubercle bacilli were made to compare their relative pathogenicity when inoculated intravenously into chickens. One strain showed the virulence of the S variant to be less pronounced than that of the R dissociate. Cultures from the organs of the chickens inoculated with the S strain yielded both R and S types of colonies. Series of inoculations of two more strains showed both the R and S variants to be identical in virulence. The S variants of the last two strains had greater pathogenic power than the R dissociates. It was impossible to say that the S variants were more virulent than their R dissociates.—*Recherches sur la virulence smooth et rough du bacille tuberculeux aviaire, N. Stamatin & V. Tomescu, Compt. rend. Soc. de biol., February 3, 1938, cxxvii, 1348.—(M. T. W.)*

Characteristics of Avian Tubercle Bacilli.—Cultures were made of organs from three tuberculous chickens on potato medium and Loewenstein's medium. Potato medium was shown to be perfectly adequate if not superior for the development of the avian type of tubercle bacilli. Further studies were made on the dissociated form of the avian type of tubercle bacillus. A particular strain of avian tubercle bacilli with its R and S variants had remained stable *in vivo* and *in vitro* for four years. Both variants were very virulent for the chicken and rabbit and even the guinea pig by intravenous inoculation. Slowly the virulence of the two variants began to change, but in a manner not equal. While both variants showed a great loss in virulence, the S type became considerably less pathogenic for the chicken, completely apathogenic for the rabbit and the guinea pig. In all cases, cultures from the organs of the infected animals yielded, on both potato and egg media, growths of the bacilli of the same variant which had been inoculated. One would think that cultures from the organs would have been more luxuriant on potato medium, but experiments made using varying dilutions of ground up organs showed the growth to be identical on each type of medium. This fact showed that dissociation could be a matter of true adaption of the strain under certain physio-chemical conditions. After several years of maintenance outside the body, the S variant might have a lower virulence than the R dissociate of the same strain. The S variant had a more limited adaption to the parasitic life.—*Sur quelque caractères de culture du bacille tuberculeux aviaire, R. Laporte & R. Schwartz, Compt. rend. Soc. de biol., June 11, 1938, cxxviii, 470.—(M. T. W.)*

Polysaccharides from Tubercle Bacilli.—Tuberculo-polysaccharides have usually been considered somewhat toxic for tuberculous guinea pigs. This toxicity is now considered to be due to the presence of traces of protein in the preparations employed as they were not free from nitrogen. The polysaccharides used in the present experiments were apparently free from all protein, hence the true response of the animal to these substances could be studied. In the first experiment, 31 tuberculous guinea pigs received injections of tuberculo-polysaccharide either into the heart or into the peritoneal cavity. Fourteen of the number were reinjected two or three weeks later. After these 45 injections there were only 4 deaths, all accidental. Two normal guinea pigs also failed to react to the material. In the second experiment the peritoneal exudates and blood cells of normal rabbits receiving bacterial and other polysaccharides were studied. Five kinds of bacterial polysaccharides, 3 from pneumococci and 2 from tubercle bacilli, in comparison with 2 simple sugars, dextrose and trehalose, as well as soluble starch given in suspension and certain diluents, distilled water, physiological saline, and Tyrode solution were used. The injections were all made intraperitoneally. In the final experiment 2 normal rabbits received daily (except Sundays) intraperitoneal injections of bovine tuberculo-polysaccharide and the effect of this on the cells of the blood and bone marrow noted. Results summarized: (*1*) Purified tuberculo-polysaccharides are relatively innocuous both to normal and to tuberculous guinea pigs. (*2*) Both tuberculo-polysaccharides and polysaccharides from pneumococci call larger numbers of leucocytes from the blood vessels than do saline and dextrose and trehalose. (*3*) The mechanisms controlling the delivery of lymphocytes and neutrophils

into the blood-stream are different. (*4*) Slight irritation of the peritoneal lining slows the delivery of lymphocytes to the blood-stream. (*5*) There are two phases in the reaction of the bone marrow to intraperitoneal injections. Correlated with the draining of neutrophils from vessels to tissues, owing to the presence of foreign materials in the latter, there is a draining of young neutrophils from the marrow into the sinuses of the marrow as these same materials reach the sinuses. The subsequent disintegration of the neutrophils extravasated into the tissues is correlated with increased myeloid activity in the marrow.—*Cellular Reactions to Polysaccharides from Tubercle Bacilli and from Pneumococci, (with one plate), F. R. Sabin, A. L. Joyner & K. C. Smithburn, J. Exper. Med., October 1, 1938, lxviii, 563.—(J. S. W.)*

Colorimetric Determination of Phthiocol.—A colorimetric method is given for estimating phthiocol in the saponified wax fractions of tubercle bacilli after separation of some of the cleavage products. Phthiocol was determined in the wax fractions of four recently isolated strains of the human tubercle bacillus. Phthiocol can be estimated also by means of the spectrophotometer, in amounts even less than 0.05 mg. in 2 per cent sodium bicarbonate solutions.—*The Chemistry of the Lipids of Tubercle Bacilli. XLIX. The Colorimetric Determination of Phthiocol, R. E. Reeves & R. J. Anderson, J. Biol. Chem., July, 1937, cxix, 543.—(F. B. S.)*

Phthiocerol in Various Strains of Human Tubercle Bacilli.—Phthiocerol in pure crystalline form was isolated only from the wax fractions of four recently isolated strains of the human type of tubercle bacilli and is, therefore, one of the characteristic metabolic products of the human type tubercle bacillus. It has been found in the wax from the bovine tubercle bacillus but not from that of the avian, timothy-grass or leprosy bacilli. Small amounts of glycerol and of carbohydrate were also found after saponification of the crude wax fractions from the four human strains of tubercle bacilli. A method for the rapid isolation of pure crystalline phthiocerol is given as follows. After the carbohydrate, higher hydroxy acids and solid and liquid fatty acids are removed from the saponified mixture, the ethereal solution is extracted with dilute sodium hydroxide, washed with water, freed from water by sodium sulfate, filtered and evaporated to dryness. After three crystallizations of the residue from ethyl acetate, rosettes of prismatic needles of phthiocerol are obtained.—*The Chemistry of the Lipids of Tubercle Bacilli. XLVIII. The Occurrence of Phthiocerol in the Wax from Various Strains of the Human Tubercle Bacillus, R. E. Reeves & R. J. Anderson, J. Biol. Chem., July, 1937, cxix, 535.—(F. B. S.)*

Phthiocerol in Bovine Tubercle Bacillus.—A dihydric, monomethoxy alcohol of the formula, $C_{35}H_{72}O_3$ or $C_{36}H_{74}O_3$ was isolated from the bovine tubercle bacillus wax. The properties of this alcohol were identical with those of phthiocerol, the characteristic alcohol of the human tubercle bacillus wax. Phthiocerol was not isolated from the timothy-grass, the leprosy or the avian tubercle bacillus. The polysaccharides of the waxes of the human and bovine tubercle bacilli were, however, entirely different and distinct.—*The Chemistry of the Lipids of Tubercle Bacilli, L. The Occurrence of Phthiocerol in the Wax of the Bovine Tubercle Bacillus, J. Cason & R. J. Anderson, J. Biol. Chem., July, 1937, cxix, 549.—(F. B. S.)*

Acetone-soluble Fat of Leprosy Bacillus.—The crude acetone-soluble fat of *Bacillus leprae* was found to be a complex mixture of free fatty acids and neutral fat. The neutral fat consisted apparently of fatty acid esters of the disaccharide trehalose and contained no glycerol. The fatty acids were composed of ordinary solid saturated acids (caproic, myristic, palmitic, stearic, arachidic, behenic, tetra cosanoic and certain unidentified new optically active higher acids), unsaturated solid acids, unsaturated liquids acids (of C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, and C_{24} series), and a series of new dextrorotatory, branched chain, saturated acids of the C_{16}, C_{15} and C_{22} series. Three different substances possessing phenolic properties were isolated.—*The Chemistry of the Lipids of Tubercle Bacilli. LII. The Composition of the Acetone-Soluble Fat of Bacillus Leprae, R. J. Anderson, R. E. Reeves & J. A. Crowder, J. Biol. Chem., November, 1937, cxxi, 669.—(F. B. S.)*

Tuberculin Protein and Polysaccharide Molecules.—This article is a description of an attempt to determine the size and other physical properties of the molecule or molecules in tuberculin which are responsible for the tuberculin reaction. Such knowledge would be valuable not alone to explain the cause of the tuberculin reaction, but in interpreting similar immunological reactions of which the tuberculin reaction is a prototype. The differences observed between the tuberculin reaction (the delayed type of reaction) and typical protein sensitization

reactions (the immediate type) may well depend on some physical property of the molecule, for both are obviously elicited by constituents in the tubercle bacillus culture filtrates. The body of the article is highly technical in nature. Part of the authors' summary follows: Studies have been made by means of sedimentation in the ultracentrifuge and by diffusion and electrophoresis, to determine the molecular weights and homogeneity of the tuberculin protein and polysaccharide molecules as found in their natural state in the unchanged filtrates from culture media after growth of tubercle bacilli. These results have been compared with data obtained on fractions isolated by chemical procedures from them or from Old Tuberculin. By means of electrophoresis in the Tiselius apparatus it was possible to separate the protein from the polysaccharide, as these two fractions occur naturally in the original culture medium filtrates of acid-fast bacilli. The protein from the bovine strain of bacillus proved to be homogeneous in sedimentation, diffusion and electrophoresis, with a molecular weight of about 10,000. The tuberculin polysaccharide isolated in electrophoresis appeared to be practically the same in sedimentation and in precipitin reaction as the polysaccharide isolated by chemical procedure. The latter proved to be homogeneous in sedimentation and diffusion with a molecular weight of about 9,000. A practically homogeneous protein was isolated from the culture filtrate of the human tubercle bacillus H37 by fractional ammonium sulfate precipitation, with a molecular weight of 32,000. It was electrochemically homogeneous, with an isoelectric point at pH 4.3 and an isoionic point at pH 4.7. It could be dried or frozen with no loss in homogeneity. It was highly antigenic in the precipitin reaction and produced the anaphylactic type of local skin reaction in tuberculous guinea pigs, in contrast to the true tuberculin type of reaction caused by a purified PPD fraction. Furthermore death resulted in tuberculous guinea pigs from intracutaneous injection of exceptionally small amounts. A protein with molecular weight of about 17,000 was isolated from the filtrate from cultures of the timothy bacillus. The nucleic acid originally occurring in Old Tuberculin seems to be responsible for the high electrochemical mobility observed. From OT and the PPD made from it, potent but nonantigenic molecules of 16,000 and 9,000 weight and with a low content of nucleic acid were isolated. With increase in size these deviated much from the normal compact spherical shape, and aggregation was evident from the tendency toward gel formation. The smallest molecule (9,000) was homogeneous and had lost some tuberculin potency.—*Molecular Weight, Electrochemical and Biological Properties of Tuberculin Protein and Polysaccharide Molecules, (with one plate), F. B. Seibert, K. O. Pedersen & A. Tiselius, J. Exper. Med., September 1, 1938, lxvii, 413.*—(*J. S. W.*)

Firmly Bound Lipids of Human Tubercle Bacillus.—The bound lipids that can be extracted from tubercle bacilli after treatment with acidified alcohol-ether vary greatly in composition, especially in respect to the carbohydrate constituent, according to the laboratory processes employed. Large quantities of polysaccharide were found in the products obtained by extracting the bacterial residues with 25 per cent alcohol or with acidified alcohol-ether. Very little of the bound lipid could be removed by means of a 50 per cent mixture of alcohol and ether, because the lipid was practically insoluble in alcohol. However, after the bacterial mass was treated with the dilute acid, the lipid was readily soluble in ether or in chloroform. This fact would indicate that the lipid is present in the cellular structure in chemical combination with some substance—either a carbohydrate or protein—but the nature of this combination remains unknown.—*The Chemistry of the Lipids of Tubercle Bacilli. LI. The Firmly Bound Lipids of the Human Tubercle Bacillus, R. J. Anderson, R. E. Reeves & F. H. Stodola, J. Biol. Chem., November, 1937, cxxi, 649.*—(*F. B. S.*)

Wax of the Bovine Tubercle Bacillus.—The purified chloroform-soluble wax from the bovine type tubercle bacillus was found to be similar in composition to the wax contained in the alcohol-ether extract of the human tubercle bacillus. On saponification the following types of compounds were found: (*a*) water-soluble constituents, glycerol and carbohydrate, (*b*) ether-alcohol-insoluble acids, containing hydroxyl and methoxyl groups and including mycolic acid, (*c*) ether-alcohol-soluble fatty acids, including a new saturated optically inactive branched chain acid ($C_{18}H_{36}O_2$) isomeric with stearic acid, and (*d*) neutral or unsaponifiable matter. The carbohydrate was a complex mixture of organic phosphoric acids and a phosphorous-containing neutral polysaccharide which on hydrolysis yielded (*a*) organic phosphoric acids, (*b*) mannose, (*c*) inosite, and (*d*) an unidentified reducing sugar. In the neutral or unsaponifiable matter was a crystalline dihydroxy monomethoxy alcohol, $C_{31}H_{72}O_3$, identical with

phthiocerol, which was found in the waxes from the human tubercle bacillus.—*The Chemistry of the Lipids of Tubercle Bacilli. LVI. The Wax of the Bovine Tubercle Bacillus, J. Cason & R. J. Anderson, J. Biol. Chem., December, 1938, cxxvi, 527.—(F. B. S.)*

Wax Fractions of the Tubercle Bacillus.—The wax-like substances extracted by an alcohol-ether mixture from the human tubercle bacillus, which are insoluble in cold acetone, were found on saponification to yield a complex mixture of the following fatty acids: (*1*) saturated normal acids such as palmitic, stearic and hexacosanoic acids (*2*) saturated branched chain acids, such as tuberculostearic, $C_{19}H_{38}O_2$, phthioic, $C_{26}H_{52}O_2$, and a levorotatory $C_{31}H_{62}O_2$ acid, (*3*) unsaturated fatty acids, belonging to the C_{26} series and on hydrogenation yielding normal hexacosanoic acid, and (*4*) the hydroxy acid, mycolic acid. Carbohydrates, glycerol and the dihydroxy alcohol phthiocerol, $C_{35}H_{72}O_3$, were present in all of the wax fractions.—*The Chemistry of the Lipids of Tubercle Bacilli. LV. Studies on the Wax Fractions of the Human Tubercle Bacillus, Charlotte W. Wieghard & R. J. Anderson, J. Biol. Chem., December, 1938, cxxvi, 515.—(F. B. S.)*

Mycolic Acid in Human Tubercle Bacilli.—The principal ether-soluble constituent of the wax of the human tubercle bacillus is a saturated hydroxy methoxy acid of very high molecular weight for which the name mycolic acid is proposed. The simplest formula calculated from the composition is $C_{88}H_{172}O_4$, assuming one hydroxyl and one methoxyl to one carboxyl group. It melts at 54° to 56°, has a specific rotation of +1.8° and is acid-fast. Decomposition products are normal hexacosanoic acid ($C_{26}H_{52}O_2$) and a non-volatile non-acidic residue.—*The Chemistry of the Lipids of Tubercle Bacilli. LIV. The Isolation and Properties of Mycolic Acid, F. H. Stodola, A. Lesuk & R. J. Anderson, J. Biol. Chem., December, 1938, cxxvi, 505.—(F. B. S.)*

Standard Sputum Requirements.—A uniform plan for the examination of sputum for tubercle bacilli is suggested. The sputum to be examined can be limited to that which is first expectorated in the morning. A plain smear of this sputum is examined on each of the first three days after admission; on the fourth day the material produced by gastric lavage is similarly examined and a culture is made of the sputum collected during the fifth day. If all of these examinations have been negative, then three twenty-four hour concentrations are done on different occasions at about weekly intervals. If the examinations have been negative for two consecutive months, a guinea-pig inoculation is done. Of course, if, at any time, during this series of examinations a positive report is returned, then the sputum is not reëxamined for one month. With patients who have no sputum the number of gastric lavage and faeces examinations would necessarily be increased. There is need for such standardization because so many patients with pulmonary tuberculosis are treated in one or more institutions. In addition, the value of clinical results will vary according to the type of sputum examination that has been carried out. It is only by means of some such scheme that one can know how many and what type of sputum examinations have been made in the months during which a particular patient is said to have had negative sputum.—*Standard Sputum Requirements, I. D. Borowitz & J. L. Leon, Quart. Bull. Sea View Hosp., July, 1938, iii, 444.—(A. A. E.)*

Concentration of Tubercle Bacilli.—Methods have been developed for improving the collection of tubercle bacilli from sputum and other body fluids. The recommended procedure involves the incorporation of alum in the sodium hydroxide used for digesting the sputum samples. When the sputum digest is neutralized, the alum flocculates and collects the tubercle bacilli. This method is as simple as any of the present methods of concentration and possesses the following advantages: (a) It reduces the centrifugation time to five minutes or, if filtration through paper is desired, dispenses with centrifugation entirely; (b) it permits the preparation of very uniform and rather thick films, thus facilitating the microscopical examination; (c) it collects bacilli more completely from the sputum or other specimens so that a unit amount of sediment contains from three to seven times more bacilli than can be collected by direct centrifugation of the same sample. In addition it has been shown that the flocculated precipitates do not interfere with the cultivation of the tubercle bacilli when very small numbers are present.—*Concentration of Tubercle Bacilli from Sputum by Chemical Flocculation Methods, J. H. Hanks, F. A. Clark & H. Feldman, J. Lab. & Clin. Med., April, 1938, xxiii, 736.—(F. G. P.)*

Concentration of Sputum.—Often tuberculous sputum contains a very small number of bacilli as determined by the ordinary methods. A higher number of positive

results are obtained by the process of homogenization as elaborated by Bezançon and Uhlenhut. By their method the sputum is converted into a homogeneous mixture which is centrifuged. The technique as described by Bezançon and Philbert consists in mixing from 2 to 3 cc. of sputum, an equal amount of lye, 20 cc. sterile water; the mixture is placed in an incubator for twenty-four hours; 5 cc. of 50 per cent alcohol is added. This homogeneous solution is centrifuged. The method has been found superior to daily successive examinations of the ground-up material by direct smear or to "homogenization" with antiformin. It is concluded that the highest number of positive results are obtained when the digested material is homogenized, then centrifuged at a speed of one thousand turns per minute for one hour.—*Rôle de la centrifugation dans la recherche du bacille de Koch par les procédés d'homogénéisation, P. E. Davy & Jean C. Levaditi, Ann. Inst. Pasteur, September, 1938, lxi, 300.*—*(J. G.)*

Cultural Methods in Diagnosis of Tuberculosis.—Cultivation is a practicable measure in the routine examination for tubercle bacilli. In a series of 3,306 specimens of all types, mainly sputa, the percentage of positives was 12.9 by direct film and 17.9 by combined film and cultural examinations. While animal inoculation is in most cases more reliable than cultivation, it is not invariably so.—*Cultural Methods in Bacteriological Diagnosis of Tuberculosis, C. A. Green, Brit. M. J., January 15, 1938, no. 4019, 111.*—*(A. P.)*

Bovine Tubercle Bacilli in Childhood and Adult Tuberculosis.—It has been shown that the bovine tubercle bacillus has a virulence for the human equal to that of the human tubercle bacillus. The bovine bacillus may cause tuberculosis in man in as many forms as the human bacillus. The results of a study covering a period of five years is presented wherein the tubercle bacillus isolated from various sources in tuberculous children and adults was typed. The bacilli were isolated by culture and guinea pig inoculation from various sources such as cerebrospinal fluid, pleural fluid, sputum, urinary sediment, gastric content, pus and material from biopsies. Loewenstein's medium with 1 per cent glycerine was used. The three following criteria were used for diagnosis of the bacilli. (*1*) If the colonies developed as eugonic and rough, the organism was diagnosed as a tubercle bacillus of the human type. (*2*) When the bacilli grew in dysgonic and smooth colonies the organisms were inoculated into rabbits to determine the type. In all instances except one when the bacilli grew in S, dysgonic colonies, the bacillus was a bovine strain. (*3*) When the bacilli were atypical in the original culture the colonies were reinoculated onto tubes of potato-glycerine culture medium or egg medium. There was a total of 843 strains isolated of which 830 were tubercle bacilli of the human type and 13 strains were bovine bacilli. Children were more often infected with the bovine bacillus than were adults, there having been 11 of the bovine strains found in children. In France tuberculosis of bovine origin even in children is minimal, especially when compared with cases reported in other countries, notably England, Scotland and Denmark. From a bacteriological standpoint it was clearly brought out from this study that in attempting to isolate tubercle bacilli of bovine origin it was essential to use ordinary medium as well as nonglycerinated medium as had previously been demonstrated by Anglo-American workers.—*Rôle de l'infection bacillaire d'origine bovine dans la tuberculose de l'enfant et de l'adulte, A. Saenz, Compt. rend. Soc. de biol., January 29, 1938, cxxvii, 269.*—*(M. T. W.)*

Bacteriology of Tuberculous Synovitis.—Two cases of infection with bovine tubercle bacilli in two butchers are reported. Both butchers were wounded in the hands in the course of their work at the abattoir. Each had been found to be positive to tuberculin before the onset of the infection with the bovine tubercle bacilli. One patient was wounded in the hand with the tooth of a beef, between the first and second phalange. An oedema was produced which spread to the neighboring finger. At the end of five months when the finger did not heal, a synovitis was diagnosed and the finger amputated. From curettage of the bone a tubercle bacillus of the bovine type was isolated. The second patient pricked himself in the thumb with a splinter of bone while at work. This produced a generalized oedema of the whole hand. A synovitis developed from which, at surgical intervention, material was obtained from which in turn bovine tubercle bacilli were grown. The first strain was obtained both from direct culture and from the spleen of a guinea pig inoculated with the synovial fluid. The colonies appeared at the end of six weeks on both glycerinated and on nonglycerinated media. The colonies were dysgonic, smooth and transparent. The bacilli isolated directly on culture from the synovial fluid

were inoculated into the meninges of a rabbit. In two weeks the animal developed a palsy of the hind quarters, which became a paraplegia three days later. The rabbit died a few days later with symptoms of meningitis. The identification of the organism was completed by intravenous inoculation of 0.001 mg. of the culture into two rabbits. One rabbit died in six weeks, the other was sacrificed in two months. Both animals showed classical lesions of generalized tuberculous infections with bovine tubercle bacilli. The second strain was obtained from three guinea pigs inoculated directly with the synovial fluid from the patient. The bacilli grew in colonies which were dysgonic and smooth and identical with the former culture. Rabbits were inoculated suboccipitally with 0.01 mg. of the culture. The rabbits became paralized in two weeks and died a few days later. Again classical tuberculous meningitis was observed at autopsy. Acid-fast organisms were found in great abundance in the granulations in the meninges at the base of the brain. Intravenous inoculations of the bacilli into rabbits produced fatal infections. The cases reported showed that a benign infection with bovine tubercle bacilli of normal virulence for laboratory animals may occur in already allergic individuals, probably having previously had a primary infection with the human type of tubercle bacillus. The degree of virulence of the secondary infection may be attributed to the certain degree of immunity already conferred by the bacilli of the primary infection. The suboccipital method of inoculation was recommended because it presents a differential diagnostic procedure of the bacillary type, because it is simple, certain and more rapid than the intravenous method of inoculation.—*Etude étiologique et bactériologique de deux cas de synovite tuberculeuse a bacille bovin, A. Saenz & D. Morel-Fatio, Compt. rend. Soc de biol., April 20, 1938, cxxvii, 1418.*—(*M. T. W.*)

Infectivity of Primary Complex.—That the lesions constituting the primary complex of tuberculosis ultimately heal, in the sense that the focus no longer contains virulent tubercle bacilli, is doubted by some. There is no unanimity of opinion concerning their infectivity in the later years of life. The problem is of clinical significance since the persistence of apparently quiescent or healed tuberculous foci within the body is a definite menace as long as virulent bacilli remain. The possibility that the bacilli might be attenuated or avirulent organisms must also be considered. In demonstrating acid-fast forms, some of which might be avirulent, it is desirable, therefore, in any investigation to both culture and inoculate portions of each specimen studied. In the present report material was obtained at autopsy from a total of 68 unembalmed bodies of human beings who had died from causes other than tuberculosis. The causes of death were as follows: malignant neoplasm 26, bacterial infection 15, cardiovascular disease 7, peptic ulcer 4, blood dyscrasia 4, pulmonary embolism 3, uraemia 3 and miscellaneous 6. The ages of these cases ranged from seven to ninety years, the largest number having been in their fourth, fifth and sixth decades. Twenty-two of the subjects were females, 46 males. Evidence of previous infection with tubercle bacilli as observed grossly was limited in most instances to well encapsulated caseous or caseocalcareous Ghon tubercles or similarly affected tracheobronchial lymph nodes. A total of 103 suspensions was prepared from the material obtained from these 68 cases and utilized for making cultures and injections. The supernatant fluid from the suspension was used directly for injecting guinea pigs and treated with dilute oxalic acid for culture purposes. The medium selected was an egg-yolk agar combination. This was prepared in two forms, one with and one without the usual glycerine, the latter for the detection of bovine bacilli. Tissues from the lesions of a majority of the subjects were also studied microscopically and evidence of activity was apparent in a considerable number. The presence of silica was demonstrated within most of the lesions. Positive results for the presence of tubercle bacilli were obtained in only one of the 68 subjects. This single subject was a fifty-four year old male who had died from injury. The material obtained for tests for infectivity consisted of one encapsulated caseocalcareous Ghon tubercle from each lung. Only the material from the right lung was positive, both on culture and inoculation. The organism obtained was of the human type of tubercle bacillus. The results of this study lead to the following conclusions: (*1*) The lesions of the primary complex of tuberculosis, when definitely encapsulated and sclerotic, or caseous or caseocalcareous, seldom contained viable or virulent organisms of *Mycobacterium tuberculosis*. (*2*) The presence or absence of viable or virulent organisms of *Mycobacterium tuberculosis* in the primary complex cannot be established by morphological appearances alone. (*3*) The data suggest that in adults endogenous reinfection is unlikely to occur from lesions

of the primary complex. (*4*) The presence of silica in varying amounts is a fairly constant finding in lesions of the primary complex of tuberculosis. In the absence of demonstrable viable tubercle bacilli in the lesions it is suggested that histological signs of activity are possibly due to silica.—*The Residual Infectivity of the Primary Complex of Tuberculosis, W. H. Feldman & A. H. Baggenstoss, Am. J. Path., July, 1938, xiv, 473.—(J. S. W.)*

Isolation of Tubercle Bacilli from Cow's Milk.—Many samples of raw milk were examined by various methods in order to determine the best way to isolate tubercle bacilli. Cultures were made of 59 samples, 41 of which were treated with 15 per cent sulphuric acid for thirty minutes. Five tubes of culture medium were inoculated with each sample. One hundred and thirty-two of the total number of inoculated tubes were contaminated with sporulating organisms, thus making the growth of tubercle bacilli impossible. Out of the 73 tubes not contaminated 2 cultures yielded tubercle bacilli pathogenic for guinea pigs. Eighteen more samples were treated with 4 per cent soda for two hours and cultured on five tubes each. Fifty-two tubes were contaminated. Of the remaining 53 tubes only one yielded a growth of acid-fast bacilli which proved to be avirulent for the guinea pig. The cultural method of milk for tubercle bacilli is thus not recommended. It was found that it required twenty-four hours for milk to be treated with a 4 per cent soda solution to free it of contaminants. Milk was artificially infected with tubercle bacilli and treated with 4 per cent soda solution. Up to four hours of action of the soda solution there was no inhibitory effect upon the tubercle bacilli. Prolongation of that time produced a bactericidal reaction upon the tubercle bacilli. The best method for isolating the bacilli from raw milk is by inoculation of the sediment from the centrifuged milk. Soda or acid treatment of milk when inoculated into guinea pigs reduced the number of deaths in the guinea pigs from secondary infection but at the same time it reduced the number of positive findings. Out of 75 samples of milk from farms in northern France 5.33 per cent were found to be infected with tubercle bacilli.—*Recherches comparatives sur les procédés d'isolement du bacille tuberculeux dans le lait de vache, R. Legrand, Ch. Gernez, P. Crampon & E. Lefort, Compt. rend. Soc. de biol., May 14, 1938, cxxviii, 204.—(M. T. W.)*

Bacillary Content of Tuberculous Material.—A method based on that of Petroff but more simple and of more practical value was used for the quantitative culturing of material infected with tubercle bacilli. For sputum, pus and all other materials containing tubercle bacilli 0.1 cc. was placed in a sterile tube to which was added 0.9 cc. of 4 per cent sodium hydroxide. This was mixed by shaking or aspirating with a pipette. If the infected material was too thick to be mixed with a pipette, bits of tissue were ground in a mortar. In both instances the mixtures were placed in the incubator for thirty minutes and shaken occasionally during that time. The mixtures were made homogeneous by aspirating with a pipette and diluted with 9 to 19 cc. of physiological salt solution to make a final dilution of 0.01 g. per cc. This was reshaken and made into serial dilutions of one-tenth to one-ten-thousandth with either physiological saline or Sauton's solution. Finally the solutions were seeded upon Loewenstein's medium, making for each specimen varying amounts of the original specimen from 1.0 to 0.000,1 mg. The tubes, two to three for each amount, were corked and placed in the incubator for two months. The cultures were examined every week and the colonies counted. Results showed an extreme richness of sputum with viable bacilli and a relative scarcity of bacilli in tuberculous pus. Each milligram of sputum was shown to contain from one to twenty thousand living bacilli, by enumeration by the method of Calmette. By this method there was a complete inhibition of secondary organisms, even when the sputum was twenty-four to forty-eight hours old. These studies showed the dangers which result from continued throwing into the atmosphere an enormous quantity of virus by people with pulmonary tuberculosis.—*Recherches sur la teneur des produits tuberculeux en bacilles de Koch, A. Boquet, Compt. rend. Soc. de biol., June 11, 1938, cxxviii, 473.—(M. T. W.)*

Bacillaemia in Tuberculosis.—Opinions as to the occurrence and frequency of bacillaemia in tuberculosis have been at great variance ever since Loewenstein's first communication on the subject in 1930. After a brief summary of the various techniques and modifications suggested by Loewenstein himself and by others, the author relates the results of a personal study on the blood of 47 patients, 27 adults and 20 children under twelve years of age. Specimens of citrated blood and of blood

treated with Loewenstein's method were planted on Loewenstein's and Petragnani's solid media and on Model's and Kirchner's synthetic liquid media, a total of eight culture tubes for each patient's specimen. After forty days, the cultures were examined microscopically and, if positive or suspicious, their material was transplanted to other tubes and at the same time inoculated into guinea pigs. Eighteen of the patients received pneumothorax treatment and the blood specimens were taken one hour after the induction of the pneumothorax and one hour after the first refill. Although some acid-resistant forms were found in several of the cultures of 4 of them, in one case only were these findings confirmed by further transplanting and guinea-pig inoculation. This was from the blood of an eight year old child who had an acute parahilar infiltration. Positive findings, confirmed in the same way, were also obtained in the cultures of 3 of the 7 patients whose blood had been taken shortly after a haemorrhage. The fact that these 3 patients showed, after the haemorrhage, disseminated lesions partly of haematogenous origin, and became progressively worse, while the 4 others rapidly recovered, leads the author to believe that the results of blood studies in such cases might be of prognostic value. Cultures from the blood of 8 pulmonary cases with extrapulmonary tuberculous complications, and of 14 children with tracheobronchial adenopathy gave negative results. In concluding, the author expresses the opinion that, though generally rare, the bacillaemia in tuberculosis may become, under special conditions, a frequent occurrence, and that the conflicting results obtained by the many investigators are due to the great difficulty of catching the short and intermittent periods during which the bacilli are present in the blood-stream.—*Ricerche sulla bacillemia nella tubercolosi polmonare, R. Cento, Lotta contro la tuberc., August, 1937, viii, 717.*—(*G. W. W.*)

Yeast-like Fungi Isolated in Pulmonary Disease.—This study was undertaken to find a rapid method of classifying monilias. The organisms used in this work were obtained from four main sources: (*1*) sputum in pulmonary diseases, (*2*) autopsy materials, (*3*) normal throats, (*4*) minor upper respiratory conditions. The results show that the reliable results of the carbohydrate utilization tests combined with precipitation and agglutination reactions furnish a dependable method of classification of these yeast-like organisms.—*Studies on Yeast-like Fungi Isolated from Pulmonary Disease, (Bronchomonilias), T. A. Keiper, J. Lab. & Clin. Med., January, 1938, xxiii, 343.*—(*F. G. P.*)

Mycological Findings in Sputum.—Specimens were studied to determine how often and what kind of fungi could be found in the sputum with and without aetiological relationship. These studies included specimens from 47 patients who had a diagnosis of pulmonary tuberculosis. Tubercle bacilli were recovered from the sputum of all of these patients. Fourteen had bronchopneumonia, 7 had asthma, 8 had bronchiectasis, 8 had sinusitis, 8 had severe colds, 2 had carcinomatous involvement of the lungs, 3 had chronic nonspecific pulmonary infections, one had undiagnosed pulmonary infection with probable malignancy, one patient had oesophageal diverticulum with possible mediastinal abscess, and 2 patients were undiagnosed until mycotic infections were discovered. The results show that seventeen patients harbored fungi in their sputum. Specimens were examined one to ten times. Yeast-like organisms were most commonly found. Coccidioides appeared three times. Two patients had double findings, *Endomyces* and *M. albicans*, and *Saccharomyces* and *Torula*. A *Sporothricum* was isolated under peculiar circumstances. Two specimens showed the presence of *Aspergilli* in their sputa over a period of two years. Fermentation tests, morphological structure and animal pathogenicity methods were used in classifying the various types of fungi isolated.—*Mycological Findings in Sputum, M. T. Woolley, J. Lab. & Clin. Med., March, 1938, xxiii, 553.*—(*F. G. P.*)

Protein Antigen from Brucella.—A purified protein was prepared from a slightly alkaline extract of *Brucella abortus* by the method used for preparing the purified protein derivative of tuberculin. A definite skin reaction lasting more than forty-eight hours can be produced when 0.005 mg. of the purified protein is injected intracutaneously in guinea pigs previously infected with different strains of brucella organisms. Larger doses may produce necrosis or even death of the animals. Sera of rabbits inoculated with *Brucella* give a positive precipitin and complement fixation tests with the antigen.—*Purified Protein Antigen from Brucella, P. Morales Otero & L. M. Gonzalez, Proc. Soc. Exper. Biol. & Med., June, 1938, xxxviii, 703.*—(*F. B. S.*)

BCG in Children.—To test the efficacy of BCG vaccine as an immunizing agent three groups of children were vaccinated subcutaneously and orally. As an index of immunity their response to the allergic properties of the vaccine was noted in their skin reactions following tuberculin tests given at stated intervals. In the first group, 655 nurslings were vaccinated orally at birth and their tuberculin reactions observed from the first month to the third year; at first every month and then, after a positive reaction, every three months. The characteristic cutaneous reaction for BCG was faint and occurred in 342 (50.6 per cent) members of this group from one month and a half to four months after vaccination. Some strong reactions were noted, occurring after the fourth month, and were attributed to superimposed infection. In the second group, 337 children were vaccinated subcutaneously. Of these, 141 were revaccinated. They had been vaccinated when nurslings and had given apparently negative reactions to all subsequent tuberculin tests. The remaining 196 children were vaccinated for the first time; 39 (19.8 per cent) developed cold abscesses one to two months after the injection. Of the 141 revaccinated children 48 (34 per cent) developed cold abscesses. The increased percentage was attributed to the phenomenon of Koch, though the allergic response as manifested by a positive cutaneous reaction to tuberculin had been present it had been too faint to be perceptible. Immunity, however, had been developed and had persisted from the first vaccination by mouth. These cold abscesses occurred more frequently in very young children, from three months to two years. Over a two-year period of observation, 69 of the 196 children, ranging from three months to twelve years, were carefully watched and 48 (68.1 per cent) gave positive tuberculin reactions. Of the 141 revaccinated ones, from one to five years old, 29 were observed and 21 (72.4 per cent) presented positive reactions. The positive reactions occurred three months after vaccination and remained positive during the two-year period. Strong reactions were noted in some and explained on the basis of superimposed infection. In the third group, 541 of 929 children vaccinated orally, between the ages of six months and fifteen years, were followed closely for three consecutive years. Of these, 265 (48.9 per cent) gave positive reactions. The negatively reacting children were revaccinated. Of these, 66 became positive giving a final total of 331 (61.1 per cent) positive reactions. The positive reactions were characteristically slight in all but three members of this group and occurred from the third to the fifth month. BCG vaccine was found to be efficacious as an immunizing agent whether given by mouth or subcutaneously. Though vaccination by the subcutaneous route was followed by a higher percentage of positive reactions the possible development of cold abscesses was a hazard. Vaccination by mouth was favored because of the facility of administration, the absence of unpleasant sequelae, the relative safety for very young children, the characteristic reaction and as demonstrated in the third group the production, by revaccination if necessary, of a percentage of positive reactions only slightly lower than that obtained by the use of the subcutaneous method.—*L'allergie chez les enfants vaccinés par le BCG., J. Stavropoulos, Arch. de méd. d. enf., September, 1938, xli, 557.*—(*A. H. C.*)

BCG Immunization.—Eleven guinea pigs received twenty daily doses of 0.25 mg. of BCG onto the conjunctiva. About six weeks later, 50 per cent of these animals showed a faintly positive reaction to tuberculin skin tests. A month later these immunized animals along with an equal number of nonvaccinated animals were given instillations onto their conjunctivae of 0.5 mg. of virulent tubercle bacilli of human type. About eight weeks later 5 vaccinated and 5 control guinea pigs were sacrificed. The nonvaccinated guinea pigs showed far more marked gross, caseated lesions of the submaxillary, cervical and tracheobronchial lymph nodes than did the vaccinated animals. The control guinea pigs showed numerous tubercles in the lungs and spleen, while the vaccinated guinea pigs showed no gross involvement of these organs, with the exception of one guinea pig in which one tubercle was found in the spleen. The remaining infected guinea pigs died at varying dates. The control animals showed gross lymph node lesions and numerous tubercles in the lungs, liver and spleen. In the vaccinated guinea pigs the submaxillary and cervical lymph nodes were caseated and often larger than in the control animals. With two exceptions, in which two or three tubercles were found in the spleen, the lungs, liver and spleen were entirely free of macroscopical lesions in the vaccinated animals. This experiment was repeated with ten more vaccinated and control animals. The results were identical. In view of these findings further experiments were carried out to determine the extent of immunization with conjunctival instillations of BCG to subcutaneous inoculations of virulent

bacilli. Immunization was carried out as in the former experiments. Eleven vaccinated guinea pigs and control guinea pigs were given subcutaneously 0.001 mg. of a virulent human strain of tubercle bacilli. The animals were sacrificed or died at varying intervals. All the control animals showed generalized tuberculosis while the vaccinated guinea pigs showed rare tubercles in the spleen and lungs or showed no macroscopical lesions in their organs. The resistance conveyed upon the guinea pig by this method of immunization was not purely local but manifested itself equally in the animals immunized in the same fashion and infected by the subcutaneous route with virulent bacilli.—*Prémunition antituberculeuse du cobaye par instillations répétées de BCG sur la conjonctive, R. Schwartz, Compt. rend. Soc. de biol., January 22, 1938, cxxvii, 205.*—(*M. T. W.*)

Dispersion of BCG in Guinea Pigs Inoculated Intravenously.—Fifteen guinea pigs were inoculated intravenously with 5 mg. of BCG and sacrificed at intervals varying from forty hours to 797 days. Cultures were made of the blood, bone marrow, liver, lungs, kidneys and spleen. The blood and bone marrow were inoculated directly onto Loewenstein's medium. The fragments of tissue of the lungs, liver and spleen and kidneys were treated with one per cent sulphuric acid for fifteen minutes, neutralized with 4 per cent sodium hydroxide and cultured on Loewenstein's medium containing malachite green. Then a second series of experiments was made in which ten more guinea pigs were inoculated intravenously with the same dosage of BCG and sacrificed five hours to 252 days later and their organs cultured as in the former experiment. The following conclusions were drawn. BCG resisted the action of sulphuric acid and permitted it to be isolated in the face of abundant contaminating microörganisms. BCG circulated in the blood-stream only for the first five hours following the inoculation. BCG was not found in the bone marrow after two weeks. BCG was irregularly found in the kidneys for a few hours. The lungs were the first organs invaded, with a massive invasion, up to the fifth day. This diminished and completely disappeared by the sixtieth day. Contrary to what Lurie found in the rabbit, BCG was more slowly fixed in the guinea pig in the liver than in the lungs. The spleen was invaded later than the lungs. This organ was found to be the most favorable for viability and the multiplication of BCG where the bacilli remained alive for ninety days. BCG penetrated into the tracheobronchial lymph nodes gradually and seemed to multiply there and remain alive longer than in the spleen. Toward the end of the first month the recrudescence appeared to be most clear. BCG had disappeared from the body of the guinea pig between the seventy-seventh to the one hundred twenty-fifth day following the inoculation.—*Sort de BCG inoculé au cobaye par voie veineuse, P. Radaody-Ralarosy, Compt. rend. Soc. de biol., April 2, 1938, cxxvii, 1192.*—(*M. T. W.*)

Immunizing Inoculations of BCG.—In order to determine how best to administer protecting doses of BCG, guinea pigs were inoculated with the vaccine in varying doses, at varying intervals and in different parts of the body. One lot of guinea pigs received 0.025 mg. of BCG four times a day for seven days, by subcutaneous injection into the skin of the abdomen, making a total of 0.7 mg. of BCG. A second lot of guinea pigs received similar inoculations but into various parts of the body. Another lot was given a single inoculation of 1.0 mg. of BCG into the skin of the abdomen. In the first lot of guinea pigs the tuberculin reaction was the most marked and was positive in a larger number of animals than in the other two groups. These lots of vaccinated guinea pigs were divided into two groups and inoculated with 0.001 mg. of a virulent strain of human tubercle bacilli at intervals of seven days apart, together with control guinea pigs, which all died with generalized inoculation tuberculosis. Four guinea pigs in the first group of animals (those which had been given repeated vaccinations at the same point) either died or were sacrificed three to four months later. The spleen, liver and lungs of these guinea pigs were entirely free of tubercles. The inguinal lymph nodes, when present, were smaller than those in the guinea pigs of the other experimental lots. In the second lot of guinea pigs (animals receiving repeated injections in different parts of the body) the guinea pigs were sacrificed about two months after the infecting inoculation. Five of these guinea pigs were entirely free of lesions. The other six showed a rare tubercle. The third lot of guinea pigs (those which had received 1.0 mg. of BCG) were sacrificed at about the same intervals and showed, on the whole, a few more tubercles, particularly in the spleen and a few more granulations in the lungs than did the animals in the other lots. Vaccinated animals of the second series (those inoculated seven days later than the first series) were sacrificed, with the exception of a few which died prematurely, about four months

after the infecting inoculation. The post-mortem findings showed about the same degree of infection as was observed in the former series. From these experiments it was learned that repeated small doses of BCG conferred more immunity upon the guinea pig than did a single large dose of BCG even when given in a larger quantity. It seemed that the animals which had received subcutaneous inoculations of BCG into different parts of the body showed a slightly higher resistance than did those which were given the same injections at one point.—*Premunition antituberculeuse du cobaye par l'immunisation "concentree" avec le BCG, R. Schwartz, Compt. rend. Soc. de biol., March 5, 1938, cxxvii, 779.—(M. T. W.)*

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